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DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study – 12-week old puppies and adults - mixed-breed dogs] – OPPTS 870.7200

PC CODES: 129121: (Fipronil); 109701: (Permethrin); 108201 (Diflubenzuron)

DP BARCODE: 419938

TEST MATERIAL (PURITY): From p. 142 of MRID 49320410: VetGuard Pro, Lot No. V.303.19, ARC Log# N13-368A; assay date: July 24, 2013. 45.20% Permethrin; 5.90% Fipronil and 3.05% Diflubenzuron. The composition of the control material (formulation blank for VetGuard Pro; considered to be Confidential Business Information) is given on p. 143 of MRID 49320410; this composition (without active ingredients) is consistent with the basic CSF (dated Feb. 18, 2014) for 89609-R. The test material is described (p. 8 of MRID 49320410) as a clear amber to light brown liquid. From information on p. 6 of MRID 49320404 (an acute oral LD_{50} study conducted at Stillmeadow) Lot No. V.303.19 had a density of 1.1496 g/mL.

TRADE NAME: VetGuard Pro Topical Solution for Dogs

CITATION: Hartwell, T. (2013) VetGuard Pro Companion Animal Safety Screen on Dogs: Final Report. Project Number: 17525/13. Unpublished study prepared by Stillmeadow, Inc. 147 p. MRID 49320410.

SPONSOR: True Science Holdings, LLC; 500 East Shore Dr., Suite 120; Eagle, ID 83616

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 49320410), there were four groups, each consisting of 12 mixed-breed dogs (3 male and 3 female adults, ages ranging from 8 to 100 months, and 3 male and 3 female puppies, ages ranging from 11.7 to 11.9 weeks). On Day -1, adults weighed 8.0-31.8 kg, and puppies weighed 5.1-9.1 kg [individual ages are reported on pages 27-28 of MRID 49320410; individual body weights are reported on pages 41-44 of MRID 49320410].

Dogs in Groups A (1X) and B (5X) were treated on day 0 with the test material; the 1X amount applied to an individual dog depended on that dog's weight (1.0 mL was a 1X dose for dogs weighing between 5.1 and 10.4 kg [11.2 to 22.9 lbs]; 2.0 mL was a 1X dose for dogs weighing from 11.0 to 17.6 kg [24.3 to 38.8 lbs]; and 4.0 mL was a 1X dose for dogs weighing 22.1 to 31.5 kg [48.7 to 69.4 lbs]). These weights and doses are consistent with the proposed label (1.0

mL for dogs weighing between 11 and 22.9 lbs; 2.0 mL for dogs weighing between 23 and 44.9 lbs; and 4.0 mL for dogs weighing between 45 and 88.9 lbs). Group A (1X dogs) were treated with one application of the test material; Group B (5X dogs) were treated with the test material applied five times (with one hour between applications). Group C dogs were treated with the placebo control (0.46X of each dog's single dose rate applied 5X at hourly intervals). Group D dogs were untreated controls.

The groups and dosages of placebo or test material, as well as mean dosages (calculated by this reviewer) of the active ingredients are given below:

Table 1

Group	Test Material	Dosage	Mean Dosage (mg/kg)					
			Permethrin		Fipronil		Diflubenzuron	
			Puppies	Adults	Puppies	Adults	Puppies	Adults
A	VetGuard Pro	1X	84.7	77.4	11.1	10.1	5.7	5.2
B	VetGuard Pro	5X	405.8	342.0	53.0	44.6	27.4	23.1
C	Placebo Control	5X	0.0	0.0	0.0	0.0	0.0	0.0
D	None	untreated	0.0	0.0	0.0	0.0	0.0	0.0

Based on the density (1.1496 g/mL) 1.0 mL of Vet Guard Pro contained 0.5196 g permethrin, 0.06783 g fipronil, and 0.0351 g diflubenzuron.

From p. 10 of MRID 49320410: "The test substance or placebo control was applied as a stripe, starting high on the back of the dog's neck to in front of the shoulder blades or as a spot between the shoulder blades. The dog was held for a few seconds to give the solution time to be absorbed into the dog's coat." [Note: the proposed label states – at least for small dogs – "deposit entire contents by squeezing the entire applicator onto dog's skin…" It is also stated: "Ensure that VetGuard Pro Topical Solution for Dogs is not applied superficially on dog's hair."]. *A clarification is needed as to whether the test material was applied directly to the skin.*

On Day 0 each dog was observed prior to each dosing and hourly at 1, 2, 3 and 4 hours after the final dose. From information on pages 49 through 52 of MRID 49320410 dogs in Groups A (1X) and D (no dosage) were observed at 0, 1, 2, 3 and 4 hours while dogs in Groups B and C (receiving 5 applications of VetGuard Pro or placebo control, respectively) were observed at 0, 1, 2, 3, 4, 5, 6, 7 and 8 hours. No observed abnormalities were noted for any of the dogs at these times.

Abnormalities observed during clinical evaluations on Day 1 were primarily cosmetic, including spiking of fur and greasiness of fur in Group A (1X) and Group B (5X). One Group A (1X) male puppy had a small circular spot of alopecia on the left thorax (no information provided as to whether this could have been dose-related or not). Two Group B (5X) adults had erythema (described in one as "slight," and in the other as "very slight"). Both of these dogs had been treated with a cumulative 20 mL of VetGuard Pro.

General health observations were conducted twice daily from Day 0 to the end of the study (the last reported observations are for AM on Day 16). The only abnormal findings were in puppies, and included ocular discharge (diagnosed as conjunctivitis) in one Group A (1X) female and one Group B (5X) male, and several sporadic occurrences of mucous and/or blood in the stool.

There were no indications of any dose-related effects involving body weight gains in puppies, body weight changes in adults, food consumption, or clinical chemistry, hematology and coagulation parameters.

There was no mortality, as all dogs survived in apparent good health.

However, the report does not provide adequate detail as to how the test material was applied (from p. 10 of MRID 49320410: "The test substance or placebo control was applied as a stripe, starting high on the back of the dog's neck to in front of the shoulder blades or as a spot between the shoulder blades. The dog was held for a few seconds to give the solution time to be absorbed into the dog's coat.") and whether or not this was consistent with label directions. The label states (for small dogs weighing between 11 and 22.9 lbs): "Deposit entire contents by squeezing the entire applicator onto dog's skin, at a single site, between the shoulder blades..." but there is no mention of direct application to the skin for heavier dogs. In addition there is an inconsistency between the study and proposed label, as the material was applied as a stripe (presumably on the heavier dogs) rather than at several spots on the dog's back (as specified on the label).

The method as to how the test material was applied (including whether it was dispensed from a pipette or other applicator) should be provided to the Agency, and an answer should be provided as to whether the test material was applied directly to the skin in this study.

Based on a 5X dosage of 0.781 mL/kg (the mean dosage for the Group B puppies) the study supports a 1X rate dosage of 0.1562 mL/kg. This is equivalent to application of 1.0 mL on a 6.40 kg (14.11 lb) dog; 2.0 mL on a 12.80 kg (28.23 lb) dog; 4.0 mL on a 25.61 kg (56.46 lb) dog and 6.0 mL on a 38.41 kg (84.68 lb) dog. A dosage rate of 1.0 mL (as stated on the label) on an 11-lb (4.99 kg) dog is equivalent to 0.200 mL/kg; 2.0 mL on a 23 lb (10.43 kg) dog is equivalent to 0.192 mL/kg; 4.0 mL on a 45 lb (20.41 kg) dog is equivalent to 0.196 mL/kg; and 6.0 mL on an 89 lb (40.37 kg) dog is equivalent to 0.149 mL/kg. A 0.200 mL/kg application rate would provide only a 3.91X [=(0.1562÷0.200) x 5] margin of safety, rather than the target 5X margin of safety indicated in the 870.7200 Companion Animal Safety Guidelines.

This companion animal safety study in adult dogs is currently classified as supplementary and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in dogs for EPA File Symbol 89609-R. It can be upgraded to acceptable to support 89609-R if 1) the registrant provides sufficient information regarding how the test material was applied and this information is consistent with product labeling and 2) if the registrant adequately addresses the 5X margin of safety issue.

COMPLIANCE: Signed and dated GLP, Quality Assurance and [No] Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: VETGUARD PRO

Description:	Described (p. 8 of MRID 49320410) as a "Clear amber to light brown liquid."
Batch #:	Lot V.303.19; ARC, Inc. for Tru Science
Purity:	45.20% Permethrin; 5.90% Fipronil; 3.05% Diflubenzuron
Storage:	At room temperature
Compound Stability:	From p. 8 of MRID 49320410: "Not provided to testing facility."
CAS #:	52645-53-1 (Permethrin); 120068-37-3 (Fipronil); 35367-38-5 (Diflubenzuron)

2. Vehicle control: VETGUARD PRO (Blank)

Description:	"Clear amber liquid."
Lot #:	V.303.23; ARC, Inc. for Tru Science
Purity:	Reported on p. 143 of MRID 49320410; its composition (without active ingredients) is consistent with the basic CSF (dated Feb. 18, 2014) for 89609-R
Storage:	At room temperature
Compound Stability:	"Not provided to testing facility."
CAS #:	Not provided

3. Test animals:

Species:	Dog
Breed:	Mixed
Age/weight (at Day 0 and/or -1)	Puppies: 11.7-11.9 months old on Day 0; day -1 weights from 5.1 to 9.1 kg (11.2 to 20.1 lbs). Adults (8 to 100 months), weights from 8.0 to 31.8 kg (17.6 to 70.1 lbs).
Source:	[From p. 9 of MRID 49320410]: Puppies from Marshall Farms; adults from Stillmeadow dog colony
Housing:	Individually housed in 3.5' x 5.5' kennels; puppies and adult dogs were in separate rooms
Diet:	[From p. 9 of MRID 49320410]: Adults received PMI 5L18 High Density Canine Diet; puppies were fed a mixture of PMI 5L18 High Density Canine Diet and Eukanuba 30/20 Premium Performance.
Water:	"Tap water, available *ad libitum*, automatic watering system."
Environmental conditions:	
Temperature:	Temperature 22°±3° C
Relative Humidity:	Relative Humidity: ~30-80%
Air changes:	19.5/hour (room containing adult dogs); 14.0/hour (room containing puppies)
Photoperiod:	12-hour light/dark cycle
Acclimation period:	[From p. 9 of MRID 49320410]: "The animals will be acclimated and observed for at least 7 days prior to treatment."

B. STUDY DESIGN:

1. **In life dates:** From p. 8 of MRID 49320410 the study was initiated on August 20, 2013, dogs were dosed on September 18, 2013 and the study termination date was October 4, 2013.

2. **Animal assignment:** From p. 10 of MRID 49320410 : "On Day -1, 24 adult dogs and 24 puppies were randomly allocated to four groups (A, B, C, and D) each comprising of 6 adult dogs, 3 males and 3 females, and 6 puppies, 3 males and 3 females." From information on p. 9 the 24 adult dogs and 24 puppies were selected from an original group of 26 adults and 26 puppies.

3. **Blinding:** There is no indication that personnel involved with data collection or the recording of observations were unaware as to the assignment of dogs to treatment groups.

4. **Dose selection rationale**: From p. 8 of MRID 49320410: "The objective of this study was to determine the adequate margin of safety of the test substance, VETGUARD PRO, following topical application to adult dogs and puppies at five times the label dose rate in accordance with US EPA OCSPP 870.7200…"

5. **Preparation and treatment**: From p. 10 of MRID 49320410: A summary of the treatment group assignments and treatments is presented in Table 2. Group A puppies and adults were treated with the test substance applied once at a single dose volume dependent on each dog's weight for a 1X dose. Group B puppies and adults were treated with the test substance applied five times, at hourly intervals, at their single dose rates, for a 5X dose. Group C puppies and adults were treated with the placebo control applied five times, at hourly intervals, at 46.11% of each dog's single dose rate. Group D puppies and adults remained untreated and served as untreated controls. The test substance or placebo control was applied as a stripe, starting high on the back of the dog's neck to in front of the shoulder blades or as a spot between the shoulder blades. The dog was held for a few seconds to give the solution time to be absorbed into the dog's coat.

Table 2 - Treatment Assignment Summary

	Group A	Group B	Group C	Group D
Number of Treatments:	1	5	5	NA
Treatment Substance:	VETGUARD PRO	VETGUARD. PRO	Placebo	Untreated
	4943-M 4953-F	4940-M 4954-F	4941-M 4957-F	4944-M 4955-F
	4946-M 4958-F	4942-M 4956-F	4945-M 4964-F	4947-M 4962-F
	4949-M 4960-F	4952-M 4961-F	4951-M 4965-F	4948-M 4963-F
	4395-M 4210-F	3517-M 4383-F	4575-M 4559-F	4338-M 4386-F
	4840-M 4650-F	4488-M 4544-F	4742-M 4607-F	4569-M 4542-F
	4846-M 4887-F	4849-M 4892-F	4851-M 4894-F	4578-M 4639-F

NA - Not Applicable; M - Males; F – Females

6. **Statistics**: From p. 11 of MRID 49320410: Statistical comparison was conducted for each parameter by day. The statistical comparison was conducted on values comparing male puppies, female puppies, male adults and female adults separately between all groups. A one-way parametric analysis of variance (ANOVA) with Tukey-Kramer's Multiple Comparisons Test was conducted on body weights, hematology and serum chemistry parameters. An unpaired t-test with Welch correction was performed on food consumption comparing pre-test food consumption (Days -7 to -1) versus post-test food consumption (Days 0 to 16).

C. **METHODS**:

1. **Observations:**

   a. **General health observations:** From p. 11 of MRID 49320410: "General health observations were conducted twice daily from Day 0 to the end of the study. General health observations included, but were not limited to, observations of general physical appearance and behavior, abnormalities of food and water consumption and appearance of urine and feces."

      The last reported observations (see pages 61-65 of MRID 49320410) are for AM on Day 16.

   b. **Physical examinations:** From p. 10 of MRID 49320410: "Complete clinical evaluations were conducted on Days -1, 1, 7 and 14. Any cosmetic effects at the dosing site were documented." The Clinical Evaluation and Physical Exam Reports (pages 29 to 32 of MRID 49320410) also include evaluations for Day -7.

2. **Body weight:** Individual body weights for days -7, -1, 7 and 14 are reported on pages 41-44 of MRID 49320410.

3. **Food consumption:** Individual food consumption data (from Day -7 through Day 16) is reported on pages 65 through 76 of MRID 49320410.

4. **Hematology and clinical chemistry**: From p. 11 of MRID 49320410: "Blood samples were collected from each animal on Days -1, 1, 7 and 14. Samples were obtained by jugular venipuncture following an overnight fast and analyzed for serum chemistry and hematology parameters." From information on p. 15 of MRID 49320410 the total amount of blood collected from each dog at each drawing was 6 mL.

   Reference ranges for hematology and serum chemistry parameters are given in Appendix A (p. 141) of the report.

   The CHECKED (X) parameters were examined.

   a. Hematology

Table 3:

X	Hematocrit (HCT)*	X	Mean corpuscular HGB (MCH)*
X	Hemoglobin (HGB)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Total leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)*
X	Erythrocyte count (RBC)*		Reticulocyte count
	Platelet count (PLT)*		
	Platelet estimate	X	Leukocyte differential count*
	Heinz bodies	X	Absolute
	Hemoglobin distribution width	X	Neutrophil
		X	Monocyte
X	Blood clotting measurements*	X	Eosinophil
X	Activated Partial Thromboplastin time	X	Basophil
X	Prothrombin time		
	Thrombin Clotting time (TCT or TT)		

*Recommended for companion animal safety evaluation based on OPPTS 870.7200

b. Clinical chemistry

Table 4

	ELECTROLYTES		OTHER
X	Calcium* (Ca)	X	Albumin*
X	Chloride* (Cl)	X	Creatinine*
	Magnesium (Mg)	X	Urea nitrogen*
X	Phosphorus * (P)		Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (Na)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, e.g., *)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total serum protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine kinase	X	Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
	Gamma glutamyl transferase (GGT)		Lipemia
	Amylase		Icterus
	Hemolysis		

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200

5. **Urinalysis:** Urinalysis was not conducted.

6. **Sacrifice and pathology**: The study did not have any necropsies as all dogs survived.

II. RESULTS

A. COMPARISON OF LABEL USE VS ADMINISTERED DOSAGE RATES:

According to the proposed label, dosages are 1.0 mL for dogs/puppies 11-22.9 lbs; 2.0 mL for dogs 23-44.9 lbs; 4.0 mL for dogs 45-99.9 lbs and 6.0 mL for dogs 89-132 lbs. The label includes the statement (under DIRECTIONS FOR USE): "Do not use on dogs that weigh under 11 lbs or are under 12 weeks of age."

In Group A (1X) each of the puppies (weight range: 5.2 to 7.7 kg, or 11.5 to 17.0 lbs) received a 1.0 mL dose; one adult female (4210-F) weighed 8.4 kg (18.5 lbs) and received a 1.0 mL dose. One adult male (4840-M) weighing 11.0 kg (24.3 lbs) and one adult female (4650-F) weighing 17.6 kg (38.8 lbs) were dosed at 2.0 mL, and the remaining 3 adults, male 4395-M weighing 27.2 kg (60 lbs), male 4846-M weighing 23.7 kg (52.2 lbs), and female 4887-F, weighing 24.3 kg (53.6 lbs) were dosed with 4.0 mL.

The following is the listing for the Group A puppies:

Table 5

Puppy Number	Amount Applied (mL)	Day -1 weight (kg)	Dosage (mL/kg)
4943-M	1.0	6.5	0.154
4946-M	1.0	6.8	0.147
4949-M	1.0	5.7	0.175
4953-F	1.0	5.2	0.192
4958-F	1.0	7.7	0.130
4960-F	1.0	5.5	0.182
			Mean = 0.163

Based on the density (1.1496 g/mL) 1.0 mL of Vet Guard Pro contains 0.5196 g permethrin, 0.06783 g fipronil, and 0.0351 g diflubenzuron. 0.163 mL/kg Vet Guard Pro would be a dosage of 84.7 mg permethrin/kg; 11.1 mg fipronil/kg; and 5.7 mg diflubenzuron/kg.

The following is the listing for the Group A adults:

Table 6

Dog Number	Amount Applied (mL)	Day -1 weight (kg)	Dosage (mL/kg)
4395-M	4.0	27.2	0.147
4840-M	2.0	11.0	0.182
4846-M	4.0	23.7	0.169
4210-F	1.0	8.4	0.119
4650-F	2.0	17.6	0.114
4887-F	4.0	24.3	0.165
			Mean = 0.149

Based on the density (1.1496 g/mL) 1.0 mL of Vet Guard Pro contains 0.5196 g permethrin, 0.06783 g fipronil, and 0.0351 g diflubenzuron. 0.149 mL/kg Vet Guard Pro would be a dosage of 77.4 mg permethrin/kg; 10.1 mg fipronil/kg; and 5.2 mg diflubenzuron/kg.

In Group B (5X) each of the puppies (weight range: 5.1 to 9.1 kg, or 11.2 to 20.1 lbs) received five 1.0 mL doses (a cumulative 5.0 mL). Three adults (4488-M, 10.1 kg or 22.3 lbs; 4383-F, 10.4 kg or 22.9 lbs, and 4544-F, 8.0 kg or 17.6 lbs) each received five 1.0 mL doses (cumulative 5.0 mL). The remaining 3 adults (3517-M, 31.5 kg or 69.4 lbs, 4849-M, 22.1 kg or 48.7 lbs, and 4892-F, 24.8 kg or 54.7 lbs) each received five 4.0 mL doses (cumulative 20 mL).

The following is the listing for the Group B puppies:

Table 7

Puppy Number	Cumulative Amount of VetGuard Applied (mL)	Day -1 weight (kg)	Dosage (mL/kg)
4940-M	5.0	6.2	0.806
4942-M	5.0	6.6	0.758
4952-M	5.0	9.1	0.549
4954-F	5.0	6.7	0.746
4956-F	5.0	5.9	0.847
4961-F	5.0	5.1	0.980
			Mean = 0.781

Based on the density (1.1496 g/mL) 1.0 mL of Vet Guard Pro contains 0.5196 g permethrin, 0.06783 g fipronil, and 0.0351 g diflubenzuron. 0.781 mL/kg Vet Guard Pro would be a dosage of 405.8 mg permethrin/kg; 53.0 mg fipronil/kg; and 27.4 mg diflubenzuron/kg.

The following is the listing for the Group B adults:

Table 8

Dog Number	Cumulative Amount of VetGuard Applied (mL)	Day -1 weight (kg)	Dosage (mL/kg)
3517-M	20.0	31.5	0.635
4488-M	5.0	10.1	0.495
4849-M	20.0	22.1	0.905
4383-F	5.0	10.4	0.481
4544-F	5.0	8.0	0.625
4892-F	20.0	24.8	0.806
			Mean = 0.658

Based on the density (1.1496 g/mL) 1.0 mL of Vet Guard Pro contains 0.5196 g permethrin, 0.06783 g fipronil, and 0.0351 g diflubenzuron. 0.658 mL/kg Vet Guard Pro would be a dosage of 342.0 mg permethrin/kg; 44.6 mg fipronil/kg; and 23.1 mg diflubenzuron/kg.

A 5X dosage of 0.781 mL/kg supports a 1X rate dosage of 0.1562 mL/kg. This is equivalent to application of 1.0 mL on a 6.40 kg (14.11 lb) dog. A dosage rate of 1.0 mL (as stated on the label) on an 11-lb (4.99 kg) dog is equivalent to 0.200 mL/kg.

B. OBSERVATIONS:

1. Observations Following Application:

From information on pages 49-52 of MRID 49320410 there were no observed abnormalities on Day 0 (post initial dose observations at 0, 1, 2, 3 and 4 hours for Group A and at 0, 1, 2, 3, 4, 5, 6, 7 and 8 hours for Groups B and C and at 0, 1, 2, 3, 4 hours for Group D.

2. Physical Examinations and Clinical Evaluations (Including Dose Site Observations):

From p. 12 of MRID 49320410: "During clinical evaluations on Day 1, abnormalities observed at the dose site included spiking of fur, greasiness of fur and erythema in Groups A and B (VetGuard Pro at 1X and 5X). *[Reviewer's note: from information on pages 33 and 34 erythema was observed in only two adults – 3517-M and 4892-F - in Group B (5X). It*

was defined as slight in 3517-M and as very slight in 4892-F. Both of these animals had been treated with a cumulative total of 20.0 mL Vet Guard Pro].

The following is from the Day 1 and Day 7 individual observations on pages 33-36 of MRID 49320410 (NOA = No observable abnormalities):

Table 9

Group		Day 1 observations	Day 7 observations
A (1X)	Puppy 4943-M	Small circular spot of alopecia on left thorax	NOA
A (1X)	Puppy 4946-M	NOA	NOA
A (1X)	Puppy 4949-M	NOA	NOA
A (1X)	Puppy 4953-F	NOA	NOA
A (1X)	Puppy 4958-F	NOA	NOA
A (1X)	Puppy 4960-F	Slight spiking of fur at dose site	NOA
A (1X)	Adult 4395-M	Slight spiking of fur at dose site	NOA
A (1X)	Adult 4840-M	Slightly greasy at dose site	NOA
A (1X)	Adult 4846-M	Slightly greasy and spiking of fur at dose site	NOA
A (1X)	Adult 4210-F	NOA	NOA
A (1X)	Adult 4650-F	NOA	NOA
A (1X)	Adult 4887-F	NOA	NOA
B (5X)	Puppy 4940-M	NOA	NOA
B (5X)	Puppy 4942-M	Fur very slightly spiked at dose site	NOA
B (5X)	Puppy 4952-M	Bilateral conjunctivitis with ocular discharge Slight spiking of fur at dose site	Ocular discharge, both eyes
B (5X)	Puppy 4954-F	NOA	NOA
B (5X)	Puppy 4956-F	NOA	NOA
B (5X)	Puppy 4961-F	Slight spiking of fur at dose site	NOA
B (5X)	Adult 3517-M	Moderate spiking, moderate greasiness and slight erythema at dose site	Moderate spiking
B (5X)	Adult 4488-M	Slight spiking at dose site	NOA
B (5X)	Adult 4849-M	Moderate spiking, moderate greasiness at dose site	NOA
B (5X)	Adult 4383-F	Very slight greasiness, slight spiking at dose site	NOA
B (5X)	Adult 4544-F	Slight erythema, slight spiking, slight greasiness at dose site	NOA
B (5X)	Adult 4892-F	Extreme spiking, moderate greasiness, very slight erythema at dose site	Slight discoloration of white fur
C (5XP)	All puppies	NOA	NOA
C (5XP)	All adults	NOA	NOA
D (untreated)	All puppies	NOA	NOA
D (untreated)	All adults	NOA	NOA

The only effects observed on Day 14 were in Group B (5X) adult 3517-M (white deposits at dose site) and in Group C (5X placebo) puppies 4957-F and 4964-F (flaky skin at dose site).

3. **General Health and Post-Dose Observations:** From p. 13 of MRID 49320410: "One male puppy from Group A had alopecia from Days 1 through 4. One female puppy from Group A had ocular discharge from both eyes on Days 5 through 7 and one male puppy from Group B had ocular discharge from Days 1 through 12. On Day 12, one female puppy in Group B had soft stool with blood and mucous. On Day 13, one male puppy from Group A had mucous in

its feces. One female puppy from Group A and one female puppy from Group C had blood in their stool on Day 13. No other abnormalities were observed for the duration of the study.

The following shows the abnormalities observed in the Day 1 through 16 individual observations, as reported on pages 53-64 of MRID 49320410:

Table 10

Group	Individual	General Health & Post-Dose Abnormalities
A (1X)	Puppy 4943-M	Small circular spot of alopecia on left thorax; Day 1 (PM observation) through Day 4 (PM observation).
A (1X)	Puppy 4953-F	Purulent ocular discharge, both eyes; diagnosis: conjunctivitis. Noted at the AM observation on Day 5; still present at the PM observation on Day 7. Treated 2X daily with Gentak on Days 6-11.
A (1X)	Puppy 4946-M	Mucous in feces; Day 13 (AM observation)
A (1X)	Puppy 4960-F	Blood in stool; Day 13 (PM observation)
B (5X)	Puppy 4952-M	Bilateral conjunctivitis with ocular discharge. Noted on Day 1 (PM) and observed through Day 12 (PM). Veterinary examination on Day 7 (AM) indicated bilateral conjunctivitis; treated 2X daily with Gentak on Days 8-13. Examination on Day 14 found the condition resolved.
B (5X)	Puppy 4961-F	Soft stool with blood and mucous; Day 12 (AM)
C (5XP)	Puppy 4965-F	Blood in stool; Day 13 (PM)

4. **Mortality:** All dogs survived to the end of the study.

C. BODY WEIGHTS:

Individual bodyweights (for days -7, -1, 7 and 14) are given on pages 41-44 of MRID 49320410. The mean weight changes (as calculated by this reviewer) for days -1 to 7 and 7 to 14 for the puppies are given below:

Table 11

Group/Sex	Mean wt. change (kg) ± SD; Days -1 to 7	Mean wt. change (kg) ± SD Days 7 to 14
Group A (1X) male puppies	0.97 ± 0.15	0.97 ± 0.35
Group A (1X) female puppies	0.63 ± 0.06	1.10 ± 0.98
Group A (1X) M + F puppies	0.80 ± 0.21	1.03 ± 0.67
Group B (5X) male puppies	0.57 ± 0.40	0.93 ± 0.81
Group B (5X) female puppies	0.83 ± 0.06	0.87 ± 0.49
Group B (5X) M + F puppies	0.70 ± 0.30	0.90 ± 0.60
Group C (5X placebo) male puppies	0.90 ± 0.40	1.03 ± 0.25
Group C (5X placebo) female puppies	0.70 ± 0.40	0.73 ± 0.67
Group C (5X placebo) M + F puppies	0.80 ± 0.37	0.88 ± 0.48
Group D (no treatment) M puppies	0.87 ± 0.50	0.20 ± 1.42
Group D (no treatment) F puppies	1.20 ± 0.30	0.93 ± 0.06
Group D (no treatment) M+F puppies	1.03 ± 0.41	0.57 ± 0.98

There is no indication from the data above that exposure to the test material had any significant effect on body weight gains in the puppies.

Mean weight changes (as calculated by this reviewer) for days -1 to 7 and 7 to 14 for the adults are given below:

Table 12

Group/Sex	Mean wt. change (kg) ± SD; Days -1 to 7	Mean wt. change (kg) ± SD Days 7 to 14
Group A (1X) male adults	-0.60 ± 0.46	-0.17 ± 0.64
Group A (1X) female adults	-0.20 ± 0.66	0.43 ± 0.68
Group A (1X) M + F adults	-0.40 ± 0.55	0.13 ± 0.67
Group B (5X) male adults	-0.77 ± 0.21	-0.07 ± 0.64
Group B (5X) female adults	0.00 ± 0.17	-0.53 ± 0.40
Group B (5X) M + F adults	-0.38 ± 0.45	-0.30 ± 0.54
Group C (5X placebo) male adults	-0.23 ± 0.55	0.17 ± 0.23
Group C (5X placebo) female adults	-0.07 ± 0.50	-0.07 ± 0.72
Group C (5X placebo) M + F adults	-0.15 ± 0.48	0.05 ± 0.50
Group D (no treatment) M adults	-0.07 ± 0.32	0.07 ± 1.11
Group D (no treatment) F adults	0.10 ± 0.20	-0.03 ± 0.25
Group D (no treatment) M+F adults	0.02 ± 0.26	0.02 ± 0.72

There is no indication from the data above that exposure to the test material had any significant effect on body weight changes in the adults.

D. FOOD CONSUMPTION:

From p. 14 of MRID 49320410 "A statistical comparison was conducted within each group upon pre-test versus post-test food consumption. Male and female puppies in Groups A, B, C and D consumed significantly more food post-test than pre-test ($p<0.0001$). All puppies were offered increasing amounts of food in order to support growth; therefore these findings do not impact the determination of the test substance's safety. Adult females in Group C ($p=0.0264$) and adult males in Group D ($p=0.0005$) consumed on average, significantly more food pre-test than post-test… Since neither of these groups were treated with the test substance, these findings do not impact the determination of the test substance's safety."

Individual daily food consumption values are reported on pages 65-76 of MRID 49320410.

Although not stated in the report, adult dogs weighing 8.0 to 11.3 kg (17.64 lbs to 24.9 lbs) usually received 250 g of dog food/day while adult dogs weighing from 17.5 to 31.8 kg (38.6 to 70.1 lbs) usually received 400 g/day. However, the data indicate one Group C (placebo) female, 4607-F, weighing between 20.0 and 20.6 kg on days -7 through 14, received 400 g on days -7 through -1, then apparently received 250 g dog food/day on days 3 through 6, 300 g dog food/day on days 7, 8, 10 and 11 (but consumed 375.7 g on day 9 out of possibly 400 g offered?) and then presumably 400 g/day days 12 through 16. No explanation is provided for these varying amounts. Group D (untreated) male 4578-M apparently received 400 g/day of food from Day -7 to -1, then 250 g from Day 3 through 6, 300 g/day from Day 7 through 11, and 400 g/day from Day 14 to 16. ***The reported statistical significances associated with post Day 0 reductions in mean food consumption in Group C (placebo) adult females and***

Group D (untreated) adult males may have involved reduced amounts of food offered to these two animals.

Puppies usually consumed all or most of the food that was offered to them. However, some individuals (Group A (1X) female 4960-F, Group B (5X) female 4961-F, and Group D (untreated) male 4948-M) frequently did not consume all of the food that was offered to them. The following are the individual daily occurrences when puppies did not consume all of the food that was offered to them in the period from day 0 to 16:

Table 13

Group	Individual Puppy	Day: Food consumption (as % of amount offered)
A (1X)	4946-M	14: 91.2%
	4949-M	9: 95.35%
	4953-F	0: 90.68%; 2: 86.2%; 3: 83.4%; 6: 69.6%;
	4958-F	15: 53.3%
	4960-F	0: 67.4%; 3: 80.9%; 5: 78.7%; 6: 48.1%; 7: 89.0%; 8: 57.3%; 9: 9.0%; 10: 86.1%; 11: 40.3%; 12: 48.4%; 13: 53.3%; 14: 66.1%; 15: 83.2%; 16: 66.3%
B (5X)	4942-M	6: 89.3%
	4952-M	9: 96.7%
	4961-F	0: 96.4%; 6: 82.5%; 7: 83.5%; 8: 71.7%; 9: 71.5%; 11: 94.0%; 13: 86.9%; 14: 83.3%; 15: 95.0%
C(5XP)	4945-M	6: 83.7%
	4951-M	0: 87.2%; 2: 94.5%; 14: 65.0%
	4957-F	2: 94.5%; 13: 86.2%; 15: 96.1%
D (0X)	4948-M	0: 96.0%; 2: 72.3%; 5: 44.2%; 6: 94.3%; 7: 59.1%; 8: 54.6%; 9: 61.1%; 10: 71.2%; 11: 52.9%; 18.4%; 14: 67.3%; 15: 89.3%
	4955-F	0: 78.0%
	4962-F	7: 86.9%

Six puppies did not eat all of the food offered on Day 0; two of these were in Group A (1X), one was in Group B (5X), one was in Group C (placebo) and two were in Group D (untreated).

It is concluded that there was no indication that treatment with the test material resulted in reduced food consumption in the puppies.

The following are the individual daily occurrences from Day 0 to Day 16 when adult dogs consumed less than 50% of the diet offered them:

Table 14

Group	Individual Adult Dog	Day: Food consumption (as % of amount offered)
A (1X)	4840-M	15: 48.5%
	4846-M	2: 38.9%
	4210-F	0: 3.5%; 1: 4.8%; 7: 29.4%;
	4650-F	0: 17.4%; 1: 14.2%; 15: 0.0%
B (5X)	4383-F	0: 43.7%; 7: 46.4%; 9: 24.2%; 15: 32.0%
	4544-F	7: 36.3%; 8: 36.4%; 14: 0%
C (5XP)	4559-F	0: 0%; 1: 12.4%; 2: 28.5%
	4607-F	0: 0%;
D (0X)	4578-M	1: 24.6%; 2: 3.3%
	4386-F	0: 24.8%; 1: 3.4%;
	4542-F	1: 46.7%

Although there were a number of cases of reduced food consumption occurring around days 0 and 1, some were in Group D (untreated controls). The "clustering" of reduced food consumption around days -1, 1, 7 and 14 may have been due to blood collections on those dates.

E. CLINICAL PATHOLOGY:

1. **Hematology:** From p. 16 of MRID 49320410 there were a small number of sporadic post-dose statistically significant observations between treated animals and their respective controls. However, these generally involved a single sex/age group, and in most cases individual values were within normal ranges. In addition, from data on pages 17-20 of MRID 49320410 there was little variation between values obtained on Days -1, 1, 7 and 14 within sex/age/dosage groups.

2. **Coagulation parameters:** From the report and information on pages 17-20 of MRID 49320410 there were no significant differences between treated animals and their respective controls.

3. **Clinical chemistry parameters:** From p. 12 of MRID 49320410: "The original draft serum chemistry results on Day 7 included values that were incompatible with life and therefore not used. Ant`ech Diagnostics repeated the tests for sodium, potassium, chloride, calcium and inorganic phosphorus and issued corrected reports for the following animals: 4949-M, 4953-F and 4650-F in Group A; 4942-M and 3517-M in Group B; 4945-M, 4951-M and 4964-F in Group C; and 4944-M, 4955-F, 4962-F and 4542-F in Group D. Due to the inconsistent results, additional blood samples were collected on Day 14."

The following is from p. 21 of MRID 49320410:

Day 1 Serum Chemistry Statistical Comparison Results

There were no significant differences in serum chemistry values for puppies or adults, male or female on Day 1 ($p>0.05$).[1]

Day 7 Serum Chemistry Statistical Comparison Results

Female puppies in Groups A, B, and C had significantly lower levels of urea nitrogen (BUN) ($p=0.0115$) and creatinine ($p=0.0132$) than female puppies in Group D. All BUN levels were within normal ranges. While the creatinine values for Groups A, B, and C are below the normal range, this is not unusual for puppies and the values are consistent with those observed on Days -1, 1 and 14. Therefore, these findings have no impact on the determination of the test substance's safety. Female puppies in Group B had levels of glucose ($p=0.0260$) and total protein ($p=0.0232$) that were significantly less than those from female puppies in Group D. The glucose levels for Group D were well above the normal range while those of Group B were normal. The total protein values for Group B were below the normal range and above the normal range for Group D. The glucose and total protein values for Group B are consistent with those observed on Days -1, 1 and 14. Therefore, these findings have no impact on the determination of the test substance's safety. Female puppies in Groups A and B had significantly less albumin levels than female puppies in Group D ($p=0.0196$).[1] All albumin values were within normal ranges; therefore, this finding has no impact on the determination of the test substance's safety. There were no other significant differences in serum chemistry values for puppies or adults, male or females ($p>0.05$).[1]

Day 14 Serum Chemistry Statistical Comparison Results

Potassium values from male puppies in the placebo control group were significantly less than potassium values from male puppies in the untreated group ($p=0.0451$). All potassium values were within normal ranges; therefore, this finding has no impact on the determination of the test substance's safety. There were no other significant differences in serum chemistry values for puppies or adults, males or females ($p>0.05$).[1]

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[1]One-way Analysis of Variance (ANOVA) with Tukey's post test was performed using GraphPad InStat version 3.06 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded [p. 26 of MRID 49320410] that:

This study was conducted to determine the safety of the test substance VETGUARD PRO, following the topical application to dogs and puppies at one and five times the label dose.

There were no abnormalities noted in any animal during the clinical evaluations on Day -1. During clinical evaluations on Day 1, abnormalities observed at the dose site included spiking of fur, greasiness of fur and erythema in Groups A and B (VETGUARD PRO at 1X and 5X). During clinical evaluations on Day 7, abnormalities observed at the dose site included spiking of fur and discoloration of fur in Group B. During clinical evaluations on Day 14, abnormalities observed at the dose site included white deposits in Group B and flaky skin in Group C (Placebo Control at 5X). Additional abnormalities observed during clinical evaluations included a small circular spot of alopecia on the left thorax of one animal on Day 1 and ocular discharge was observed in another animal on Days 1 and 7. Abnormalities noted during general health and post-dose observations included thin appearance, alopecia, ocular discharge, soft stool with blood and mucous, mucous in feces and blood in stool. There were no significant differences in body weight or body weight change for puppies or adults, males or females. Male and female puppies in Groups A, B, C and D consumed significantly more food post-test than pre-test. Adult females in Group C and adult males in Group D consumed on average, significantly more food pre-test than post-test.

There were various significant differences in the serum chemistry and hematology values among groups, but there were no trends that could be determined and the differences were not consistent between days, groups, sex, or age of animals.

VETGUARD PRO was safe to apply to adult male and female dogs and to male and female puppies at the label dose and at five times the label dose.

B. REVIEWER'S COMMENTS AND CONCLUSIONS:

This reviewer concurs that there was no indication of any significant toxicity in adult male and female dogs and 12-week old puppies following dosage at 1X and 5X of what was termed "the label dose" of VetGuard Pro.

However, the report does not provide adequate detail as to how the test material was applied (from p. 10 of MRID 49320410: "The test substance or placebo control was applied as a stripe, starting high on the back of the dog's neck to in front of the shoulder blades or as a spot between the shoulder blades. The dog was held for a few seconds to give the solution time to be absorbed into the dog's coat") and whether or not this was consistent with label directions. The label states (for small dogs weighing between 11 and 22.9 lbs): "Deposit entire contents by squeezing the entire applicator onto dog's skin, at a single site, between the shoulder blades…" but there is no mention of direct application to the skin for heavier dogs. In addition there is an inconsistency between the study and proposed label, as the

material was applied as a stripe (presumably on the heavier dogs) rather than at several spots on the dog's back (as specified on the label).

The protocol as to how the test material was applied (including whether it was dispensed from a pipette or other applicator) should be provided to the Agency, and an answer should be provided as to whether the test material was applied directly to the skin in this study.

Based on a 5X dosage of 0.781 mL/kg (the mean dosage for the Group B puppies) the study supports a 1X rate dosage of 0.1562 mL/kg. This is equivalent to application of 1.0 mL on a 6.40 kg (14.11 lb) dog; 2.0 mL on a 12.80 kg (28.23 lb) dog; 4.0 mL on a 25.61 kg (56.46 lb) and 6.0 mL on a 38.41 kg (84.68 lb) dog. A dosage rate of 1.0 mL (as stated on the label) on an 11-lb (4.99 kg) dog is equivalent to 0.200 mL/kg; 2.0 mL on a 23 lb (10.43 kg) dog is equivalent to 0.192 mL/kg; 4.0 mL on a 45 lb (20.41 kg) dog is equivalent to 0.196 mL/kg; and 6.0 mL on an 89 lb (40.37 kg) dog is equivalent to 0.149 mL/kg. A 0.200 mL/kg application rate would provide only a 3.91X [=(0.1562÷0.200) x 5] margin of safety, rather than the target 5X margin of safety indicated in the 870.7200 Companion Animal Safety Guidelines.

The registrant should address the 5X margin of safety, and can take into consideration the amount of test material that is actually released by 1.0 mL (and other size) applicator tubes. However, it may be necessary to revise (increase) the minimum dog weights or reduce the dosages specified in the proposed label.

This companion animal safety study in adult dogs is currently classified as supplementary and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in dogs for EPA File Symbol 89609-R. It can be upgraded to acceptable to support 89609-R if 1) the registrant provides sufficient information regarding how the test material was applied and this is consistent with product labeling and 2) if the registrant adequately addresses the 5X margin of safety factor.